Sleep spindles as a diagnostic and therapeutic target for chronic pain

Bassir Caravan[1], Prathamesh Kulkami[2], Lizbeth Hu[3], Daniel Veyg[3], Zhe S. Chen[2,4,5,*], Jing Wang[3,4,5,*]

1. New York University School of Medicine, New York, NY 10016
2. Department of Psychiatry, New York University School of Medicine, New York, NY 10016
3. Department of Anesthesiology, Perioperative Care and Pain, New York University School of Medicine, New York, NY 10016
4. Department of Neuroscience & Physiology, New York University School of Medicine, New York, NY 10016
5. Neuroscience Institute, New York University School of Medicine, New York, NY 10016

*To whom correspondence should be addressed: Z.S.C. (zhe.chen@nyulangone.org) and J.W. (jing.wang2@nyulangone.org)

Abstract

Pain is known to disrupt sleep patterns, and disturbances in sleep can further worsen pain symptoms. Sleep spindles occur during slow wave sleep have established effects on sensory and affective processing in mammals. A number of chronic neuropsychiatric conditions, meanwhile, are known to alter sleep spindle density. The effect of persistent pain on sleep spindle waves, however, remains unknown, and studies of sleep spindles are challenging due to long period of monitoring and data analysis. Utilizing automated sleep spindle detection algorithms built on deep learning, we can monitor the effect of pain states on sleep spindle activity. In this study we show that in a chronic inflammatory pain model in rodents, there is a significant decrease in sleep spindle activity compared to controls. This suggests that sleep spindles may serve a role in the development of much-needed noninvasive and objective biomarkers for pain states. Methods to restore sleep spindles, meanwhile, is associated with decreased pain symptoms. These results suggest that sleep spindle density correlates highly with chronic pain and may thus be both a potential biomarker for chronic pain and a target for neuromodulaton therapy.

Introduction

Chronic pain affects one in four adults worldwide, and diagnosis currently relies on self-reported verbal pain assessments. Reliable and useful assessment of pain are crucial to the diagnosis and effective management of pain. Therefore, the development of objective biomarkers can revolutionize our ability to understand and treat pain. Biomarkers that demonstrate disease mechanisms are particularly useful, as they are more likely to be reproducible across distinct populations, and may guide therapies.

Poor quality sleep has been shown to be a risk factor for chronic pain,[1] with numerous studies implicating sleep in pain-associated depression and anxiety as well.[2] A causal link between chronic pain and sleep deficits, however, is not well established. Nevertheless, there are reports

that suggest interventions during sleep may have potential benefits in treating pain.[3] Thus, objective measurements of sleep quality present a promising diagnostic and therapeutic target for pain.

Sleep spindles are bursts of high frequency (11-16 Hz) oscillations of neural activity that occur during stage 2 non-rapid eye movement (NREM) sleep. While spindle waves are generated by the thalamus, they are relayed through thalamocortical oscillations to the cortex. Spindles have been well studied in mammalian models.[4] It has been shown that spindles have functions in both memory consolidation and sensory processing.[5,6] Deficits in spindle activity during sleep have also been linked to numerous pathologies including epilepsy and other neuropsychiatric illness.[7,8] Recent studies have shown that spindles are involved in the attenuation of signals from the external environment, suggesting a role for spindle waves in processing nociceptive inputs.[9]

The challenge of studying sleep spindles is the labor-intensive process of spindle identification and characterization. However, with recent advances in automated detection of sleep spindles using neural network algorithms, [10,11] it is possible to study sleep spindle activity as a possible biomarker for certain health states. In this study, we apply a recently developed deep learning method, known as SpindleNet, to characterize sleep spindle activity in chronic pain-treated rats. Our results indicate that sleep spindle density is decreased in a model of chronic inflammatory pain. Further, we show that pink noise, an established method to increase spindle density, can effectively decrease pain sensitivity in this animal model.

Results

Each rat slept for a minimum of 1 hour in the sleep recording apparatus for every 3 hours of experimentation (Figure 1). Sleep vs. wake state was classified based on the video and accelerometer measurement of rat's movement (Fig. 2a, b), and such classification was used to search for sleep spindles given the EEG signal (Figure 2c). Overall, sleep duration did not differ significantly between experimental groups, recording days, or experiment sessions.

A deep neural network, SpindleNet, has been recently developed to automatically detect spindle episodes in real time (Figure 3). The output of SpindleNet provided soft thresholds for the detection of spindles (Figure 3b), which were left consistent across all rats and sessions to allow correlations across time. SpindleNet rejected any spindles that did not meet the minimum duration criterion (1 s?? for rats) necessary to be considered a spindle episode. Detected spindles that met our pre-determined probability threshold and the minimum duration criterion were counted for the duration of the sleep session. We computed the relative spindle density measures by the ratio of the number of isolated spindles (detected by SpindleNet) per minute of sleep (exclusively for NREM sleep). Our SpindleNet can be easily integrated with any neuromodulation method in open- or closed-loop stimulation.

Our first research question is to investigate the impact of chronic pain on sleep spindles. We used Complete Freund's Adjuvant (CFA) to induce persistent inflammatory pain in the hind paws. We found that CFA-treated rats, compared with saline-treated rats (controls), showed a marked reduction in spindle density during sleep one day after the onset of pain. We correlated these

relative spindle densities with behavior by measuring the mechanical nociceptive threshold, and we found that rats with increased allodynia have reduced spindle activity during sleep (Figure 4). As rats recovered from CFA, spindle density returned to baseline or the pre-CFA levels.

In contrast, control rats showed no symptoms of mechanical allodynia, nor did they show changes in spindle density over the time course of the experiment. We observed an inverse correlation between relative spindle activity and allodynia (Figure 4c), indicating a close relationship between sleep spindle density and persistent pain. These results also suggest that spindle density may be a biomarker for the severity of pain.

Our next research question is to investigate whether acoustic stimulation can affect sleep spindles and chronic pain symptoms. To dissect a possible causal relationship between pain symptoms and sleep deficits, we analyzed if restoration of sleep spindles could relieve pain. Pink noise, a type of low frequency acoustic stimulation, is a well-known method to enhance sleep spindles.[12-16] Thus, we introduced random pink noise based on a Gaussian random distribution with average interval between stimuli of 10 seconds. Rats subject to such noise showed an immediate intra-session increase in spindle density (Figure 5). Detected sleep spindles had an average frequency between 10-15 Hz with variable duration (Figure 5d). There was no statistically significant difference in the shape of spindles in the context of pink noise and spindles in the absence of pink noise, with both sets of spindles exhibiting the same frequency, duration, and power distributions. We further examined the effect of pink noise on pain symptoms. In order to maximize the treatment effects of acoustic stimulation, we exposed rats to 14 consecutive days of acoustic stimulation. We used spared nerve injury (SNI) to model stable chronic pain. SNI-treated rats were exposed to 14 consecutive days of pink noise stimulation during 8 hours of sleep in a quiet room. We measured allodynia response before and after the pink noise treatment regimen, and observed a statistically significant change in behavioral pain response as manifested by relief of mechanical allodynia (Figure 6). Together, these results indicate that rhythmic acoustic stimulation, by elevating spindle density, could reduce chronic pain symptoms.

Commented [WU1]: does this mean there is no effect of pink noise on spindle density?

Discussion

The appropriate assessment of pain, particularly chronic pain, is essential to understanding pain mechanisms and providing proper treatment. Current pain assessment in both humans and animal models relies on subjective verbal or behavioral reports. However, the development of objective neurophysiological measures can complement subjective behavioral measures to yield promising advances in the diagnosis and management of pain.

All sensory information received from the external environment, including nociception, passes through the thalamus before reaching the cortex. The thalamus, through its interaction with the cortex, has a long established role in attention and sensory gating mechanisms during sleep.[17] [REF: McCormick and Bal, 1994; Chen et al., 2016]. Sleep spindle waves occur during NREM sleep, and they have been shown to play a role in learning, memory consolidation and sensory processing.[5,6,18] Meanwhile, deficits in spindle activity during sleep have also been linked to numerous pathologies including epilepsy, altered mentation, poor memory retention, and other neuropsychiatric illness.[7,8] Interestingly, recent studies have also demonstrated the role for sleep

spindles may be in the attenuation of signals from the external environment, as a form of sensory gating.[9] This role in sensory gating suggests the involvement for spindle waves in processing nociceptive inputs as well.

A number of studies have demonstrated a relationship between spindle density and sleep stability, with the underlying mechanisms being perturbations in thalamocortical oscillation.[19,20] At the same time, an increasing number of studies have also shown that chronic pain is associated with poor sleep quality, especially interruptions in slow wave, NREM sleep (**MORE REFS**).[1] This is not surprising, given the role for thalamocortical dysrhythmia in chronic pain.[21-23] Since sleep spindle density has been implied in many functional roles in memory consolidation and sensory gating, it is important to accurately identify and characterize sleep spindles. Our SpindleNet can serve as an excellent spindle detector due to its robust performance[10]. Our study makes the link between these two lines of inquiries. We found that depressed spindle waves are associated with chronic pain. Due to the role of spindles in sensory gating, cognition, and affect, through modulations of thalamocortical oscillation, it is quite plausible that deficits in spindle waves would contribute to the pathology or pathogenesis of chronic pain. Our finding that acoustic stimulation exposure can enhance spindle waves and over time decrease allodynia in rats with chronic pain provides further support for this hypothesis.

The use of EEG findings as a biomarker for neuropsychiatric diseases has gained considerable interest over the last decade. Compared with other neuroimaging modalities such as the MRI, EEG is cheaper, more portable and easier to use. Modern source localization has further improved the spatial resolution for high-density EEG studies, advancing the specificity and sensitivity of EEG biomarkers[24,25]. Acute pain triggers characteristic evoked potentials (or event related potentials, ERPs) on the EEG, as well as elevated theta and gamma powers. More importantly, the power of theta and gamma oscillations are altered in chronic pain state, and changes in these EEG nociceptive response have been shown to predict the presence of pain and analgesic effects[26-31]. Further, recent machine learning-driven feature extractions of EEG signals have been shown to distinguish pain patients and healthy controls[32]. These studies support the use of EEG as a potential tool to derive biomarkers or biosignatures for pain. In this study we have demonstrated an unique use of spindle activity during sleep as an EEG finding for pain states. Sleep spindles can be measured easily at home or in the hospital, and can be used to track the presence and progression of pain symptoms. Future work in humans can validate the use of spindle density changes as a biomarker for chronic pain.

Our results also have therapeutic implications, as our strategy to enhance spindles is associated with decreased allodynia. Acoustic stimulation is non-invasive, and much research has been conducted in the use of acoustic stimuli to manipulate spindles, with multiple studies showing measurable behavioral impacts on memory and cognition in human subjects.[7,12,33,34] However, protocols for spindle manipulation vary, with some groups utilizing bursts of white or pink noise as in this study, and others even implementing closed-loop electrical or acoustic stimulation in real-time.[35,36] It remains to be seen if random acoustic stimulation is as effective as closed-loop acoustic stimulation, suggesting further study is required in exploring optimal spindle manipulation paradigms, especially with regards to impact on behavior. Since sleep spindles are often

temporally coupled with slow waves, it also remains to be explored for developing optimized stimulation strategy (timing and duration) for alleviating chronic pain symptoms.

In summary, we have applied a deep neural network approach to automate the analysis of sleep spindles in rodents. Our study indicates that spindle density is decreased in the chronic pain state. Acoustic stimulation, meanwhile, can enhance spindles and relieve chronic pain symptoms. Therefore, our study suggests the potential for sleep spindles to function as targets for pain diagnosis and for therapeutic neuromodulation.

Materials and Methods

Rats

Experiments were conducted on male Sprague-Dawley rats obtained from Taconic Farms, Albany, NY. All procedures in this study were approved by the New York University School of Medicine (NYU SoM) Institutional Animal Care and Use Committee (IACUC) as consistent with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals to ensure minimal animal use and discomfort. Animals were given on average 14 days to acclimate to the facility prior to experimentation.

EEG Surgery

Rats were put under anesthesia (isoflurane, 1.5-2%) and placed in a stereotactic frame before undergoing implantation. A midline incision of 4 cm was made and several small screw-based electrodes were anchored on superficial holes drilled on the skull pertaining to PFC (Bregma +3.5 mm, midline) and bilateral hind-limb S1 (Bregma -2 mm, 2 mm lateral) regions. Screws were soldered to female Mill-Max connectors, and ground and reference screws were anchored above the cerebellum. Dental cement was used to secure equipment with bone screws. Rats were allowed to recover for at least 1 week after surgery.

Sleep measurements

Sleep studies were performed over 4-5 hours in a quiet, isolated room in ambient temperature during the typical day cycle. The timing of recording was consistent throughout the course of study. Each rat was acclimated to sleep testing conditions in their home cage. Intan RHD2000 boards were connected to the rat at the start of the experiment and two video cameras recorded the rat for the duration of the experiment. Each rat received one week of acclimation, two days of baseline recordings, and experimental conditions were split between control and pain models. The pain model used was Complete Freund's Adjuvant (CFA).[37] After baseline recordings, rats received CFA injection in the hind paw, and their sleep was measured 1, 3, and 7 days after injection.

Analysis

After isolating sleep using manual labeling of video, EEG time series acquired at 1 kHz were bandpass filtered and downsampled to 200 Hz then fed into a deep neural network (SpindleNet) to automatically detect spindle episodes. Spindle density was calculated as a measure of the

number of spindles detected per minute of sleep recorded and normalized by average baseline recording data to calculate relative spindle density over time and compare across rats.

Allodynia testing

Mechanical allodynia testing was performed using the Dixon up-down method with von-Frey filaments.[38,39] Rats were placed on a mesh table, allowed to acclimate, and the hind limb paw was stimulated with filaments of logarithmically increasing stiffness. Withdrawal thresholds were recorded and 50% mechanical nociceptive threshold was computed, as described previously.[40]

Pink noise stimulation

The stimuli were bursts of pink 1/f noise of 50ms duration, with a 5-ms rising and falling time, respectively. Pink instead of white noise was used because it sounds softer and is therefore more comfortable to hear. Pink noise stimulation was performed using a computer speaker at fixed volume located near the rat in the controlled sleep environment. The noise file was generated by Audacity's built-in pink noise generator. Stimulation frequency was normalized from a gaussian distribution to a mean of 10 seconds between noises with a standard deviation of 3.33 seconds. Noise experiments were conducted at the end of a regular sleep recording session.

Spared Nerve Injury

Rats underwent a spared nerve injury (SNI) procedure, as described previously.[40,41] During the procedure, rats were anesthetized (isoflurane 2%). An inferomedial incision was made from the left knee to expose the sciatic nerve bundle. The branches of the sciatic nerve were identified, and the tibial and peroneal nerves were severed and tied off, sparing the sural nerve. Rats were allowed to recover for at least 1 week after surgery.

Sleep staging

Video and accelerometer measurements of the rat's movement were used to classify sleep vs wake states. Among sleep periods, non-rapid eye movement (NREM) sleep was primarily determined by the high delta/theta EEG power ratio and the presence of slow waves and sleep spindles.

Automatic spindle detection

We used a previously established method, known as SpindleNet, to detect sleep spindles from a single-channel EEG.[10] SpindleNet is a deep learning-based method for online detection of sleep spindles. SpindleNet consists of a convolution neural network (CNN) and recurrent neural network (RNN). It was operated and run on single-channel downsampled (200 Hz) sleep EEG signals. Compared to other state-of-the-art detection methods, SpindleNet can achieve low detection latency and high detection accuracy and specificity. In addition, SpindleNet can produce robust performance with various EEG sampling frequencies and signal-to-noise ratio (SNR). SpindleNet was run on a Linux computer with an embedded Nvidia GPU card.

Acknowledgments

This research was partially supported by the NSF grant CBET-1835000 (Z.SC., J.W.), NIH grants R01-NS100065 (Z.S.C.,J.W.) and R01-MH118928 (Z.S.C.).

References

1 Choy, E. H. The role of sleep in pain and fibromyalgia. *Nat Rev Rheumatol* **11**, 513-520, doi:10.1038/nrrheum.2015.56 (2015).

2 Ojeda, B. *et al.* Understanding the different relationships between mood and sleep disorders in several groups of non-oncological patients with chronic pain. *Curr Med Res Opin* **34**, 669-676, doi:10.1080/03007995.2017.1384372 (2018).

3 Gerhart, J. I. *et al.* Relationships Between Sleep Quality and Pain-Related Factors for People with Chronic Low Back Pain: Tests of Reciprocal and Time of Day Effects. *Ann Behav Med* **51**, 365-375, doi:10.1007/s12160-016-9860-2 (2017).

4 De Gennaro, L. & Ferrara, M. Sleep spindles: an overview. *Sleep Med Rev* **7**, 423-440 (2003).

5 Ulrich, D. Sleep Spindles as Facilitators of Memory Formation and Learning. *Neural plasticity* **2016**, 1796715, doi:10.1155/2016/1796715 (2016).

6 Marshall, L., Helgadottir, H., Molle, M. & Born, J. Boosting slow oscillations during sleep potentiates memory. *Nature* **444**, 610-613, doi:10.1038/nature05278 (2006).

7 Landis, C. A., Lentz, M. J., Rothermel, J., Buchwald, D. & Shaver, J. L. Decreased sleep spindles and spindle activity in midlife women with fibromyalgia and pain. *Sleep* **27**, 741-750, doi:10.1093/sleep/27.4.741 (2004).

8 Ferrarelli, F. & Tononi, G. The thalamic reticular nucleus and schizophrenia. *Schizophr Bull* **37**, 306-315, doi:10.1093/schbul/sbq142 (2011).

9 Claude, L. *et al.* Sleep spindles and human cortical nociception: a surface and intracerebral electrophysiological study. *The Journal of physiology* **593**, 4995-5008, doi:10.1113/JP270941 (2015).

10 Kulkarni, P. M. *et al.* A deep learning approach for real-time detection of sleep spindles. *Journal of neural engineering* **16**, 036004, doi:10.1088/1741-2552/ab0933 (2019).

11 Lustenberger, C. *et al.* High-density EEG characterization of brain responses to auditory rhythmic stimuli during wakefulness and NREM sleep. *NeuroImage* **169**, 57-68, doi:10.1016/j.neuroimage.2017.12.007 (2018).

12 Papalambros, N. A. *et al.* Acoustic Enhancement of Sleep Slow Oscillations and Concomitant Memory Improvement in Older Adults. *Frontiers in human neuroscience* **11**, 109, doi:10.3389/fnhum.2017.00109 (2017).

13 Choi, J., Han, S., Won, K. & Jun, S. C. The Neurophysiological Effect of Acoustic Stimulation with Real-time Sleep Spindle Detection. *Conference proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual Conference* **2018**, 470-473, doi:10.1109/EMBC.2018.8512323 (2018).

14 Ngo, H. V., Claussen, J. C., Born, J. & Molle, M. Induction of slow oscillations by rhythmic acoustic stimulation. *J Sleep Res* **22**, 22-31, doi:10.1111/j.1365-2869.2012.01039.x (2013).

15 Ngo, H. V. *et al.* Driving sleep slow oscillations by auditory closed-loop stimulation-a self-limiting process. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **35**, 6630-6638, doi:10.1523/JNEUROSCI.3133-14.2015 (2015).

16 Ong, J. L. *et al.* Effects of phase-locked acoustic stimulation during a nap on EEG spectra and declarative memory consolidation. *Sleep Med* **20**, 88-97, doi:10.1016/j.sleep.2015.10.016 (2016).

17 McCormick, D. A. & Bal, T. Sensory gating mechanisms of the thalamus. *Current opinion in neurobiology* **4**, 550-556 (1994).

18 Halassa, M. M. *et al.* State-dependent architecture of thalamic reticular subnetworks. *Cell* **158**, 808-821, doi:10.1016/j.cell.2014.06.025 (2014).
19 Wimmer, R. D. *et al.* Sustaining sleep spindles through enhanced SK2-channel activity consolidates sleep and elevates arousal threshold. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **32**, 13917-13928, doi:10.1523/JNEUROSCI.2313-12.2012 (2012).
20 Dang-Vu, T. T., McKinney, S. M., Buxton, O. M., Solet, J. M. & Ellenbogen, J. M. Spontaneous brain rhythms predict sleep stability in the face of noise. *Current biology : CB* **20**, R626-627, doi:10.1016/j.cub.2010.06.032 (2010).
21 LeBlanc, B. W. *et al.* T-type calcium channel blocker Z944 restores cortical synchrony and thalamocortical connectivity in a rat model of neuropathic pain. *Pain* **157**, 255-263, doi:10.1097/j.pain.0000000000000362 (2016).
22 Leblanc, B. W., Lii, T. R., Silverman, A. E., Alleyne, R. T. & Saab, C. Y. Cortical theta is increased while thalamocortical coherence is decreased in rat models of acute and chronic pain. *Pain* **155**, 773-782, doi:10.1016/j.pain.2014.01.013 (2014).
23 Walton, K. D., Dubois, M. & Llinas, R. R. Abnormal thalamocortical activity in patients with Complex Regional Pain Syndrome (CRPS) type I. *Pain* **150**, 41-51, doi:10.1016/j.pain.2010.02.023 (2010).
24 Kaiboriboon, K., Luders, H. O., Hamaneh, M., Turnbull, J. & Lhatoo, S. D. EEG source imaging in epilepsy--practicalities and pitfalls. *Nature reviews. Neurology* **8**, 498-507, doi:10.1038/nrneurol.2012.150 (2012).
25 Fischer, I. W. *et al.* Objective methods for the assessment of the spinal and supraspinal effects of opioids. *Scand J Pain* **14**, 15-24, doi:10.1016/j.sjpain.2016.10.001 (2017).
26 Diers, M. *et al.* Central processing of acute muscle pain in chronic low back pain patients: an EEG mapping study. *J Clin Neurophysiol* **24**, 76-83, doi:10.1097/01.wnp.0000241093.00844.0e (2007).
27 Flor, H., Braun, C., Elbert, T. & Birbaumer, N. Extensive reorganization of primary somatosensory cortex in chronic back pain patients. *Neuroscience letters* **224**, 5-8 (1997).
28 Grosen, K. *et al.* Predictors of opioid efficacy in patients with chronic pain: A prospective multicenter observational cohort study. *PloS one* **12**, e0171723, doi:10.1371/journal.pone.0171723 (2017).
29 Gram, M., Graversen, C., Olesen, A. E. & Drewes, A. M. Machine learning on encephalographic activity may predict opioid analgesia. *European journal of pain* **19**, 1552-1561, doi:10.1002/ejp.734 (2015).
30 Prichep, L. S., Shah, J., Merkin, H. & Hiesiger, E. M. Exploration of the Pathophysiology of Chronic Pain Using Quantitative EEG Source Localization. *Clin EEG Neurosci* **49**, 103-113, doi:10.1177/1550059417736444 (2018).
31 Tiemann, L. *et al.* Behavioral and neuronal investigations of hypervigilance in patients with fibromyalgia syndrome. *PLoS One* **7**, e35068, doi:10.1371/journal.pone.0035068 (2012).
32 Brown, C. A., Almarzouki, A. F., Brown, R. J. & Jones, A. K. P. Neural representations of aversive value encoding in pain catastrophizers. *Neuroimage* **184**, 508-519, doi:10.1016/j.neuroimage.2018.09.052 (2018).
33 Sato, Y., Fukuoka, Y., Minamitani, H. & Honda, K. Sensory stimulation triggers spindles during sleep stage 2. *Sleep* **30**, 511-518, doi:10.1093/sleep/30.4.511 (2007).
34 Leminen, M. M. *et al.* Enhanced Memory Consolidation Via Automatic Sound Stimulation During Non-REM Sleep. *Sleep* **40**, doi:10.1093/sleep/zsx003 (2017).
35 Lustenberger, C. *et al.* Feedback-Controlled Transcranial Alternating Current Stimulation Reveals a Functional Role of Sleep Spindles in Motor Memory Consolidation. *Current biology : CB* **26**, 2127-2136, doi:10.1016/j.cub.2016.06.044 (2016).

36 Ngo, H. V., Martinetz, T., Born, J. & Molle, M. Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron* **78**, 545-553, doi:10.1016/j.neuron.2013.03.006 (2013).
37 McCarson, K. E. Models of Inflammation: Carrageenan- or Complete Freund's Adjuvant (CFA)-Induced Edema and Hypersensitivity in the Rat. *Curr Protoc Pharmacol* **70**, 5 4 1-9, doi:10.1002/0471141755.ph0504s70 (2015).
38 Chaplan, S. R., Bach, F. W., Pogrel, J. W., Chung, J. M. & Yaksh, T. L. Quantitative assessment of tactile allodynia in the rat paw. *Journal of neuroscience methods* **53**, 55-63, doi:0165-0270(94)90144-9 [pii] (1994).
39 Bourquin, A. F. *et al.* Assessment and analysis of mechanical allodynia-like behavior induced by spared nerve injury (SNI) in the mouse. *Pain* **122**, 14 e11-14, doi:S0304-3959(05)00535-X [pii]
10.1016/j.pain.2005.10.036 (2006).
40 Wang, J. *et al.* A single subanesthetic dose of ketamine relieves depression-like behaviors induced by neuropathic pain in rats. *Anesthesiology* **115**, 812-821, doi:10.1097/ALN.0b013e31822f16ae
00000542-201110000-00033 [pii] (2011).
41 Decosterd, I. & Woolf, C. J. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* **87**, 149-158, doi:S0304-3959(00)00276-1 [pii] (2000).

McCormick DA, Bal T. Sensory gating mechanisms of the thalamus. Curr. Opin. Neurobiol. 4, 550-556. (1994)

Chen Z, Wimmer RD, Wilson MA, Halassa MM. Thalamic circuit mechanisms link sensory processing in sleep and attention. Front. Neural Circuits 2016

Astori S, Wimmer RD, Luthi A. Manipulating sleep spindles--expanding views on sleep, memory, and disease.

Trends Neurosci. 2013 Dec;36(12):738-748.

Antony JW, Paller KA. Using oscillating sounds to manipulate sleep spindles. Sleep, 40, zsw068 2017)